UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte WILLIAM EDWARD EVANS
and OLIVER GENE MCDONALD

Appeal No. 2005-1163
Application No. 09/829,113

ON BRIEF

Before SCHEINER, GRIMES, and GREEN, Administrative Patent Judges.

GRIMES, Administrative Patent Judge.

DECISION ON APPEAL

This appeal involves claims to a method of determining whether a set of two variable sequences are present on the same chromosome in a DNA sample. The examiner has rejected the claimed method as obvious in view of the prior art. We have jurisdiction under 35 U.S.C. § 134. We reverse.

Background

1. Haplotypes

Human cells contain two copies of each chromosome and therefore two copies of every gene. Each copy of a gene can contain sequence variations that may (or may not) affect the encoded amino acid sequence and may (or may not) affect the function of the

encoded protein. Such sequence variations are called polymorphisms. Appellants also refer to them as "nucleotide polymorphisms" or "NPs". See, e.g., the specification at page 3.

The claims on appeal are directed to a method of determining the haplotype of a gene that might contain two polymorphisms. "Haplotype" refers to the condition of a single copy of the gene in the chromosome; the claimed method determines whether two known polymorphisms are present in one of the two copies of a chromosomal gene.

The specification provides an example of why this can be important. See pages 16-18. The TPMT gene apparently encodes an enzyme that metabolizes thiopurine medications. The TPMT gene exists in polymorphic forms: "About 90% of individuals inherit high TPMT activity, 10% intermediate activity due to heterozygosity at the TPMT locus, and 1 in 300 inherit TPMT deficiency. . . . These patients are at high risk for severe hematopoietic toxicity [when given thiopurines]. . . . However, these toxicities can be avoided if thiopurine dosages are decreased 90-95%." Specification, page 17.

These polymorphisms are due to mutations in the TPMT gene. The members of one set of such mutations are known as TPMT*3A, -*3B, and -*3C. "Heterozygotes who inherit one of these mutant alleles (*3A, *3B, *3C) and one wildtype allele (*1) have intermediate TPMT activity, and intermediate intolerance to thiopurine therapy. . . . Compound heterozygotes, with a TPMT*3B/*3C genotype, one allele containing only [a first] mutation and the other containing only [another] mutation, would be TPMT-deficient. Thus, it is critical to determine whether the individuals who are heterozygous at both [of the mutated sites] have these mutations on the same (*1/*3A

genotype) or different (*3B/*3C genotype) TPMT alleles, as their risk of toxicity and thiopurine dosages will be markedly different." Specification, page 18.

2. PCR and its variants

The polymerase chain reaction (PCR) is a method for amplifying a desired fragment of DNA. See, e.g., the Appeal Brief, page 6, note 2. In PCR, primers are made that flank the desired DNA and a series of reaction steps are repeated: double-stranded DNA in the sample is made single-stranded, the primers are allowed to hybridize, and an enzyme creates a new second strand starting with each of the primers. The products of each reaction cycle serve as new targets for subsequent amplification steps. PCR thus allows scientists to make any number of copies of a desired DNA.

Numerous variants of PCR that have been developed, including "allele-specific PCR" and "inverse PCR". In allele-specific PCR, the primers are designed to amplify only one of two (or more) possible alleles. Alleles are polymorphic (variant) forms of a gene that coexist within a population. See the specification, page 5, line 24, to page 6, line 8. The enzyme used in PCR will only create a second DNA strand starting with a primer in which the last nucleotide is hybridized to the target DNA. See Patel, page 3561, right-hand column ("if a PCR primer has a mismatch at its 3'-terminal with regard to the template, the efficiency of extension by Taq polymerase will be reduced.").[1] The primers used in allele-specific PCR are designed to hybridize to, and amplify, only a single specific allele.

[1] Lo et al., "Direct haplotype determination by double ARMS: specificity, sensitivity and genetic applications," Nucleic Acids Research, Vol. 19, pp. 3561-3567 (1991). Both the examiner and Appellants refer to this reference as "Patel", so we will, too.

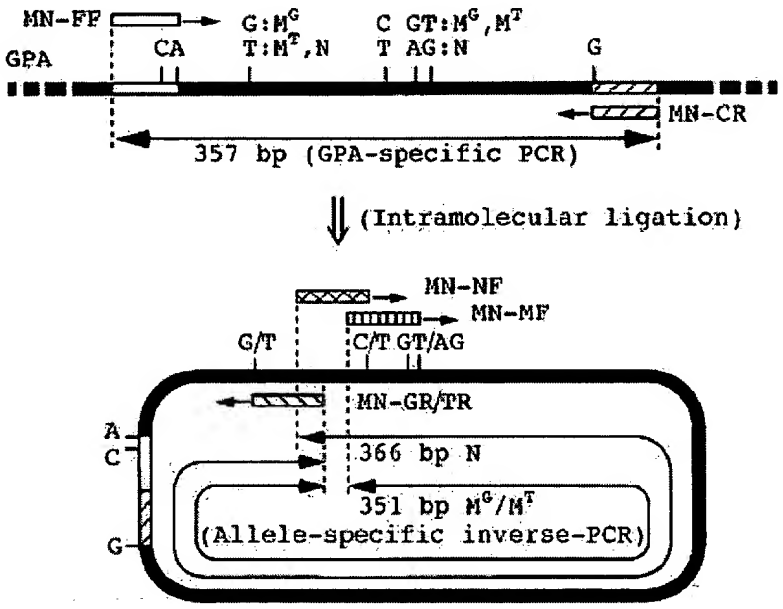
Inverse PCR has been used "to analyze unknown sequences that flank a region of known sequence." Li, page 360, right-hand column.[2] "This technique . . . involves ligation of separated regions at the ends of a sequence (a restriction fragment or a PCR product)." Id. That is, a fragment of DNA – generated either by conventional PCR amplification or by cutting a larger DNA molecule with a restriction enzyme – is circularized by attaching (ligating) the two ends of the fragment to each other. Unlike in conventional PCR, the primers used in inverse PCR are oriented away from each other (hence the "inverse" in inverse PCR). Therefore, second-strand synthesis begins at one primer and goes around the length of the circularized DNA fragment until it reaches the second primer.

Finally, allele-specific inverse PCR, or ASIP, combines the features of allele-specific PCR and inverse PCR. Like inverse PCR, the DNA fragment being amplified is generated from an earlier round of conventional PCR, is circularized before the ASIP procedure is carried out, and uses primers that are oriented away from each other. Like allele-specific PCR, ASIP uses primers that are designed to hybridize to, and amplify, only one of two alleles that could be present at a given location.

---

[2] Li et al., "Allele-specific, inverse-PCR amplification for genotyping MN blood group," BioTechniques, Vol. 25, pp. 358-361 (1998).

Li's Figure 1 provides an illustration of allele-specific inverse PCR:[3]



The figure shows that a fragment of DNA (357 bp of the GPA gene) is amplified by a first, conventional PCR process. That fragment is circularized by intramolecular ligation. The circularized DNA is then used for allele-specific inverse PCR: one of two primers is used to prime DNA synthesis in one direction (to the right in the figure). The primers are designed so that each will support synthesis of only one of the two polymorphisms (known as M and N) that might be present.[4] When the M-specific primer (MN-MF) is used and the DNA fragment contains an M polymorphism, a 351 bp fragment will be amplified; if the M polymorphism is not present, no DNA synthesis takes place. The N-specific primer (MN-NF) likewise supports PCR amplification and produces a 366 bp fragment only if the N polymorphism is present.

---

[3] The figure shown here has been redacted slightly.
[4] The M allele includes both the "M^G" and "M^T" shown in the figure. The difference between the two is not important to the present discussion, but will be addressed later.

Discussion

1. Claim construction

Claim 1, the broadest claim on appeal, reads as follows:

> 1. A method for determining the haplotype structure of a contiguous DNA segment comprising a first nucleotide polymorphism (NP) and a second NP separated by at last 200 nucleotides, said method comprising:
>
> (a) obtaining a DNA sample comprising said contiguous DNA segment;
>
> (b) using said DNA sample as a template for polymerase chain reaction (PCR) amplification of a DNA fragment comprising said contiguous DNA segment,
>
> wherein the PCR amplification is performed with
>
> a first primer capable of annealing to a region adjacent to the first NP and distal to the second NP and
>
> a second primer capable of annealing to a region adjacent to the second NP and distal to the first NP;
>
> (c) ligating the ends of said DNA fragment to each other so as to produce a circular DNA molecule, wherein said first NP and said second NP are brought into closer proximity on said circular DNA molecule relative to said contiguous DNA segment; and
>
> (d) determining the haplotype of said first NP and said second NP.

Thus, claim 1 is directed to a method for determining the haplotype of a DNA segment that contains two polymorphisms separated by at least 200 base pairs. The claimed method comprises obtaining a DNA sample and carrying out a conventional PCR procedure: amplifying the DNA segment containing the polymorphisms using two primers that each hybridize (a) adjacent to one of the polymorphisms and (b) on the side of the polymorphism away from (distal to) the other polymorphism. Thus, the PCR amplification begins outside one of the polymorphisms, includes all of the 200-plus base pairs in between the polymorphisms, and ends outside the other polymorphism.

The claimed method also requires that, after amplification, the ends of the amplified DNA fragment ("said DNA fragment") are ligated to circularize it, "wherein [the two polymorphisms] are brought into closer proximity on said circular DNA." The final step in the claimed method is "determining the haplotype" by any appropriate method.

2. Obviousness

The examiner rejected claims 1-16, 21, and 22 under 35 U.S.C. § 103 on the basis that the claimed subject matter would have been obvious in view of Li, Patel, and Michalatos-Beloin.[5] The examiner's position is that the method disclosed by Li differs from that of claim 1 only in that the polymorphisms in Li's DNA fragment are separated by less than 200 base pairs. See the Examiner's Answer, page 6. See also id., page 10 ("This invention is a form of ASIP (allele specific, inverse PCR), in which the only difference from the standard ASIP method is the distance between the first and second nucleotide polymorphism.").

The examiner reasoned that "Patel teaches that inverse PCR methods such as those used by Li can be applied to haplotype sequences up to 10 kb apart," and that "Michalatos-Beloin teaches haplotyping methods where the molecules are prepared by long range PCR . . . [and that] amplification of up to 40 kb should be possible." Examiner's Answer, pages 6 and 7. He therefore concluded that it would have been obvious to apply Li's method to samples in which two polymorphisms are separated by more than 200 base pairs.

[5] Michalatos-Beloin et al., "Molecular haplotyping of genetic markers 10 kb apart by allele-specific long-range PCR," Nucleic Acids Research, Vol. 24, pp. 4841-4843 (1996).

As a basis for motivation, the examiner relies heavily on the following statement in Li: "Although these alleles can by typed be allele-specific nested PCR following GPA-specific PCR, ASIP, rather than nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR." See the Examiner's Answer, page 7 (citing Li, page 361, column 1).

We note that the examiner's analysis is at odds with our interpretation of the claimed method. The examiner interprets the claims to be directed to "a form of ASIP (allele specific, inverse PCR), in which the only difference from the standard ASIP method is the distance between the first and second nucleotide polymorphism." We disagree. The claimed method lacks the features of either allele-specific PCR or inverse PCR. Unlike inverse PCR, the claimed method involves primers directed towards, not away from, each other. Unlike allele-specific PCR, the only primers required in the claimed method amplify the target fragment regardless of which allele is present at each of the polymorphic positions. Granted, these techniques could be involved in the final step of the claimed method ("determining the haplotype"), but neither is required by claim 1.

Appellants argue that the references do not support a prima facie case of obviousness because, among other things, when Li circularizes the PCR-amplified DNA (as shown in Figure 1), the polymorphisms are the same distance apart as they were in the genomic DNA. Thus, Appellants argue, the reference does not suggest the claimed method, which requires that the two polymorphisms be "brought into closer proximity on said circular DNA."

The examiner has asserted that, in Li, "the ligation brings the first and second polymorphisms into closer proximity on the circular DNA molecule (see figure 1[])]." Examiner's Answer, page 5. The examiner did not, however, provide any further explanation of why he considered Li's figure to support his view. As we interpret the figure, it shows the polymorphic nucleotides to be separated by the same distance before and after circularization. We therefore agree with Appellants that Li does not teach this aspect of the claimed method.

Apparently as a backup position, the examiner argues that "[e]ven if Li does not exemplify bringing polymorphisms closer together, Li expressly teaches and suggests such a course, and expressly teaches methods to analyze haplotypes whose polymorphisms are too far apart for standard PCR." Examiner's Answer, page 15. As we understand it, the examiner's position again relies on the final sentence in Li: that "ASIP, rather than nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR."

Appellants argue that the examiner has misconstrued this sentence and that, when Li's disclosure is considered in context, the reference provides no motivation to apply the disclosed method to DNA having two polymorphisms spaced more than 200 base pairs apart or to carry out a ligation that brings two polymorphisms closer together. See the Appeal Brief, pages 9-11. Appellants have provided a declaration under 37 CFR § 1.132 to support their interpretation of Li.

We agree with Appellants that when Li is considered in its entirety, it does not suggest the instantly claimed method. Li teaches that the M and N red blood cell antigens are encoded by the glycophorin A (GPA) gene. Page 358, left-hand column.

Whether the GPA gene encodes the M or N antigen is determined by nucleotide polymorphisms at three specific locations: the M antigen has the bases C, G, and T at the three positions, while the N antigen has T, A, and G, respectively. Li discloses that, at a different location in the GPA gene, the N antigen has the base T and the M antigen can have either T or G. Li refers to an M antigen with a T in this position as M^T and one with a G as M^G. See page 358, left-hand column, and Figure 1 (reproduced on page 5, above).

Li teaches that "M^G, M^T and N alleles can be typed by RFLP [restriction fragment length polymorphism] or SSCP [single-strand conformation polymorphism] analysis but not by the single ASPA [allele-specific PCR amplification] technique." Page 358, left-hand column. A single allele-specific PCR amplification could not be used because "[a]lthough primers specific to M^G and N alleles can be designed, the M^T- (and N-) specific nucleotide T . . . is located too far (30-43 bp upstream) from the three M- (M^G and M^T) specific bases . . . to design a single M^T-specific primer." Page 358, middle column. That is, a single primer could not be used to distinguish the M^T allele from both M^G and N because such a primer would have to span the 30-43 bases in between the M^T-specific T nucleotide and the three nucleotides that distinguish M from N; evidently, primers used in allele-specific PCR cannot be that long.

Li teaches that allele-specific inverse PCR can be used to distinguish the M^G, M^T, and N alleles. That is, Li designed a set of primers (MN-NF and MN-MF) to distinguish between the M and N alleles and designed a second set of primers (MN-GR and MN-TR) to distinguish between the G and T nucleotides that are characteristic of the M^G and N/M^T alleles respectively. Using these primers in different combinations shows

whether the sample contains the M^T-, M^G-, or N-specific combination of polymorphisms. See the legend to Figure 2: "Lanes marked G show amplification of 351-bp M^G allele using primers MN-MF and MN-GR. T lanes show amplification of 351-bp M^T and/or 366-bp N allele(s) using primers MN-MF, MN-NF and MN-TR."

Li concluded that "[i]nverse PCR . . . can also be applied to closely linked polymorphisms. . . . [U]sing inverse PCR, the linked polymorphisms can be analyzed by a single procedure using allele-specific primers. In this study, M^G, M^T and N alleles were regarded as haplotype alleles and analyzed by ASIP." Paragraph bridging pages 360-361. In this context, Li stated that "[a]lthough these alleles can be typed by allele-specific nested PCR following GPA-specific PCR, ASIP, rather than nested PCR, can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR." Id.

We agree with Appellants that the examiner has misinterpreted this statement. Read in context, what Li is saying is that after the relevant section of the GPA gene is amplified by conventional PCR, the M^G, M^T, and N alleles can be distinguished by either allele-specific inverse PCR (as used by Li) or by the alternative technique of "allele-specific nested PCR", even though the nucleotides that distinguish the three alleles are too far apart to allow the use of plain old allele-specific PCR.

Li's statement that "ASIP . . . can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR" does not have the meaning the examiner has given it. It is not a suggestion to carry out the disclosed procedure on a DNA in which two polymorphisms are too far apart to allow the DNA between them to be amplified by conventional PCR (and therefore necessarily more

than 200 bp apart). That interpretation of Li does not square with the rest of Li's disclosure. Li's method relies on a first step of amplifying a stretch of DNA that contains each of the polymorphisms of interest and all of the intervening DNA. Since PCR amplification is a required part of Li's method, Li cannot reasonably be read to suggest using the method to haplotype polymorphisms that are too far apart to be amplified by PCR.

In addition, Li's allele-specific inverse PCR method involves amplifying, in an allele-specific manner, the part of the previously amplified DNA between the polymorphisms of interest. See Figure 1. Again, Li discloses nothing to suggest that this technique would work if the polymorphisms of interest were too far apart to allow the DNA between them to be amplified by PCR.

The examiner has not explained how his interpretation of Li would fit with the rest of Li's disclosure. The concluding statement of Li makes sense only in the context of the earlier explanation that single-step allele-specific PCR cannot be used to distinguish the alleles because the M^T/N-specific nucleotide is located too far from the M/N specific nucleotides. That is, Li's concluding statement that "ASIP . . . can be applied to haplotyping of polymorphisms separated by a distance that is too long to be amplified by PCR" means that ASIP can be used to haplotype polymorphisms that are too far apart to be amplified by the usual allele-specific PCR technique, not polymorphisms that are too far apart to be amplified by PCR at all.

In our view, Li does not suggest the aspects of the claimed method requiring the polymorphisms to be at least 200 base pairs apart and to be brought closer together when the amplified DNA is circularized. The examiner has pointed to nothing in Patel or

Michalatos-Beloin that suggests these limitations. The cited references therefore do not support a prima facie case of obviousness and the rejection of claims 1-16, 21, and 22 is reversed.

The examiner also rejected claim 17 as obvious in view of Li, Patel, Michalatos-Beloin, and Krynetski,[6] and rejected claim 18 as obvious in view of Li, Patel, Michalatos-Beloin, and Martin.[7] Krynetski and Martin, however, were cited only for suggesting application of the method of claim 1 to specific genes. Their disclosures do not remedy the deficiencies discussed above. The rejections of claims 17 and 18 are reversed.

Other Issues

As noted above, we disagree with the examiner's position that the claimed method differs from that of Li only in the distance separating the polymorphisms. As we interpret the claims, in fact, the closest prior art is provided by Patel.

Patel discloses an experiment (pages 3565-3566) in which the haplotype of two polymorphisms that are apparently separated by 1154 – 919 = 235 bp is determined by "double ARMS inverse PCR." Patel teaches that by circularizing a DNA fragment and re-opening the ring at a different site, "genomic targets [i.e., polymorphisms] which are far apart could be brought close enough together for inverse PCR to be carried out." Page 3565. The re-linearized DNA is then assayed to determine whether the polymorphisms are present. See Figure 7.

---

[6] Krynetski et al., "A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase," Proc. Natl. Acad. Sci. USA, Vol. 92, pp. 949-953 (1995).
[7] Martin et al., "SNPing away at complex diseases: Analysis of single-nucleotide polymorphisms around APOE in Alzheimer disease," Amer. J. Human Genet., Vol. 67, pp. 383-394 (2000).

Patel does not disclose whether, in the DNA fragment used, the polymorphisms actually end up closer to each other than they were before. The disclosed method also differs from the method of instant claim 1 in that the DNA circularized was apparently not derived from a PCR reaction but from restriction enzyme treatment of genomic DNA. See page 3562, left-hand column, first paragraph ("Ten ml of blood were collected. . . . DNA extraction was carried out."), and id., last two paragraphs ("One µg of genomic DNA was cut with 6 units of Sau3AI. . . . The restricted DNA was then ligated . . . to promote the formation of monomeric circles. . . . The circularised DNA was then relinearised by digestion with 5 units of Rsa I.").

On return of this application, we encourage the examiner to re-evaluate the teachings of Patel in view of the claim construction discussed in this opinion and the knowledge of those skilled in the art as of the application's effective filing date. If the examiner is of the view that the differences between the instantly claimed method and the method disclosed by Patel would have been obvious to a person of ordinary skill in the art, our decision regarding the rejection on appeal does not foreclose a rejection based on Patel and other references that may suggest the limitations distinguishing the claimed method from Patel's.

Summary

We reverse the rejection of claims 1-18, 21, and 22 under 35 U.S.C. § 103 because the cited references would not have suggested the instantly claimed method to a person of ordinary skill in the art.

REVERSED

Toni R. Scheiner)
Administrative Patent Judge)
)
)
Eric Grimes) BOARD OF PATENT
Administrative Patent Judge) APPEALS AND
) INTERFERENCES
Lora M. Green)
Administrative Patent Judge)

EG/dym

Alston and Bird LLP
St. Jude Children's Research Hospital
Bank of America Plaza
101 South Tryon Street, Suite 4000
Charlotte, NC 28280-4000